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## **SURFACE MODIFICATION FOR BIOCOMPATIBILITY**

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## FIGURE CAPTIONS

- Figure 1: Panel of phase images of E18 rat cortical neuron cultures after 4 days in culture in MEM + N3/g + 5 ng/ml bFGF on APTS monolayers either modified by apotransferrin (APO) or bovine serum albumin (BSA) adsorbed to the surface or derivatized with covalent crosslinker, glutaraldehyde (GLU). Controls are shown here: poly-D-lysine (A), APTS (B), and APTS + GLU (C).
- Figure 2: Panel of phase images of E18 rat cortical neuron cultures after 4 days in culture in MEM + N3/g + 5 ng/ml bFGF on APTS monolayers either modified by apotransferrin (APO) or bovine serum albumin (BSA) adsorbed to the surface or derivatized with covalent crosslinker, glutaraldehyde (GLU). APTS + APO (A); APTS + GLU + APO (B); APTS + BSA (C); APTS + GLU + BSA (D). Controls were APTS, APTS + GLU, and poly-D-lysine (see Fig. 1).
- Figure 3: Fluorescent images of cells attached to the substrate APTS, modified by adsorbed BSA after 15 days in culture. Expression of microtubule associated protein- MAP 2 (A) and glial fibrillary acidic protein- GFAP (B) indicate survival of both neuronal and non neuronal cells on these substrates.
- Figure 4: Phase (A) and fluorescent (B) images of E18 rat cortical neuron cultures on substrate APTS covalently linked via GLU to BSA, after 15 days in culture, stained with monoclonal to GFAP.

## PROJECT SUMMARY

The aim of this work is to create surfaces on implantable silicon microstructures for the purpose of controlling the interaction of neurons, glia, and related cells and their protein products with the microstructure. A major effort in previous work was devoted to the examination of culture conditions for early embryonic cultures with an aim to promote longevity in culture. We have established a serum-free culture system that approximates the composition of cerebral spinal fluid (CSF) for culturing the embryonic cells and have now extended this work to adult cultures as well. For embryonic cells, the E16 cortical cultures survive to 21 days and the E19 cells survive to 19 days. Adult cultures have been examined for up to 14 days *in vitro*.

In this quarter we have focussed on repeating experiments with adult, E 14, and microglial cultures for publication. We have also extended our investigations of techniques for producing biologically modified substrates as well as screening of these substrates in embryonic culture (E18). We continue to examine the impact of substrate on neuronal subpopulations and neurotransmitter production (E14), in addition to impact on non-neuronal cell expression. Because we have established a reproducible *in vitro* model for cortical neurons from E14 to adult cells as well as the other cell types contained in the CNS such as astrocytes and microglia we can reliably test the effect of surfaces, factors, and insult in a reproducible defined system before *in vivo* experiments.

We have furthered our plans for *in vivo* implantation studies by formulating experimental approaches for the examination of materials, substrates, cell response and analysis with Dr. Cordell Gross at University of Vermont to start in this next quarter.

## OBJECTIVES

Overall project objectives:

- a) Selecting candidate surfaces that are likely to enhance the microscopic mechanical stabilization of a microstructure implanted within the central nervous system;

- b) Selecting candidate organic surfaces that are likely to enhance the close approximation of neurons or neuronal processes to specific regions of implanted silicon microstructures;
- c) Developing or adapting available methods to bond the selected organic molecules to a silicon dioxide surface like the surface of a micromachined electrode (Tanghe and Wise, 1992) and to chemically characterize these surfaces before and after protein adsorption.
  - 1. The attachment method shall be stable in saline at 37°C for at least 3 months;
  - 2. To use silane coupling as the method of attachment;
  - 3. To use the silanes to control the spatial extent (i.e., the pattern) of the deposited surface.
- d) Developing a cell culture or other suitable model of mammalian cortex and investigate the growth and adhesion of neurons, glia, micro-glia, and other cells present in the nervous system on substrates coated with the selected surfaces;
- e) Cooperating with other investigators in the Neural Prosthesis Program by coating microelectrodes (estimated 50 over the contract period) with the most promising materials for *in vivo* evaluation as directed by the NINDS Project Officer.

#### QUARTER OBJECTIVES

- Continue work to establish adult cortical cell culture conditions
- Begin screening biologically modified SAM surfaces for cortical cell response
- Continue surface analysis of surfaces both before and after culture
- Continue surface stability experiments
- Continue screening surfaces for microglia response
- Send samples to Huntington for *in vivo* experiments as well as explore other possibilities for collaboration in this area.

## BACKGROUND

Biomaterials that penetrate into the central nervous system as the microscopic electrode shafts of neural prostheses interact with neural and other tissues on a cellular and molecular level. In order to achieve tight coupling between these implanted microelectrodes and the target neural substrate, this interaction must be understood and controlled. Controlling the interaction requires an understanding of how cells, including neurons and glia, and extracellular proteins respond to the surface chemistry and any leachable substances of implanted biomaterials. This contract supported research will study these interactions with a long-term goal of rationally designing microelectrode surfaces to promote specific tissue interactions.

Presently, available clinical neural prosthetic implants typically use stimulus levels that excite volumes of neural tissue ranging from cubic millimeters to cubic centimeters around the electrode. Because of the large stimulus intensities required, precise control of the response of neurons within the first few cell layers of an implanted electrode has not been necessary. Recent developments in the areas of micromachining and fabrication of silicon integrated circuit microelectrodes have introduced the possibility of controlled stimulation of smaller volumes of neural tissue--on the order of one thousand to one hundred thousand times smaller than those used today.

The efficiency of these microelectrodes depends on the micro-environment around stimulating sites. The surface of the microelectrodes and the proteins that adsorb to this surface have a major impact on the way in which different cell populations react to the implant. Neural growth cones are sent out from many neurons around a microelectrode following implantation. With appropriate surfaces it may be possible to get selected neurons to send processes directly to the microelectrodes. Glia and other cells also respond to an implanted electrode. With appropriate surfaces it may be possible to promote cell adhesion and anchoring of some areas of the implant structure while leaving other areas with minimal response from glial cells. This study will investigate cellular and molecular responses to specific surface modifications of silicon microelectrodes.

## RESULTS

### *Surface Analysis and Stability Measurements*

X-ray photoelectron spectroscopy (XPS) is necessary for this program in the same way that an NMR spectrometer is necessary for conducting an organic synthesis program. Since we are synthesizing surfaces and modifying their properties, we will need to assay the result of the surface before (starting material) and after (reaction product) modification. This is analogous to examining a procedure by NMR. One would not think to run an organic synthetic reaction without an examination of the product at the end of each step; in the same sense, it is crucial for us to examine the product in our surface modification experiments.

Previous work in this contract has shown that the adsorbed proteins expected in any real biological evaluation stabilize the SAM monolayer for an initial period. The reaction mechanism of the SAM with the surface is also a key element, and we have seen groups with multiple attachment points (monochloro vs. trichloro silanes) to be more stable also. Finally, the glass itself appears to be unstable in the presence of PBS; so we are now extending these experiments to other surfaces used in making microelectrodes and their passivation layers.

As touched upon in the last report, we have been studying techniques to examine and concentrating on the production of a variety of adsorbed and covalently linked biomolecules on SAMs . We have surface analytical results that prove the viability of our modification protocol to attach biomolecules to surfaces via a covalent cross linker and are now concentrating on examining longterm survival as well as protein deposition and cell population and sub-population expression. Figures 1,2 (A-G) show the results of an experiment examining adsorbed and covalently linked biomolecules apo-transferrin (APO) and bovine serum albumin (BSA) to the SAM, APTS, in cultures of F18 cultures in MEM + N3/g in the presence of 5 ng/ml bFGF. While the outcomes are preliminary and subtle: survival, process outgrowth and cell population expression are modified by the presence of proteins: both absorbed and covalently linked BSA (2 C, D) and covalently linked APO (2 A, B) as early as day 4. Later examination of cultures at day 15, with the use of antibodies to

glial fibrillary acidic protein( GFAP) and microtubule associated protein 2 (MAP 2) indicate the survival of mixed populations of cells (Figure 3 a, b; 4a, b). We are in the process of analyzing the results of this experiment with XPS and will present these results in later reports.

We have extended the production and examination of substrates modified with cell substrate adhesion proteins, such as N-CAM, laminin, peptides, GAGs and other proteins and anticipate cell culture results on these in this next quarter.

#### *Surface modification and cell culture*

In the last quarter we successfully established cultures of adult cortical neuron cells in our defined *in vitro* model system. This quarter we extended our studies of adult cultures for up to 14 days. We will extend this work to further the goals of the program in determining the best surfaces to derivatize microelectrodes for *in vivo* applications. In this next quarter we will specifically examine the electrophysiology nature and health of the adult neurons in combination with screening of new materials/developing compatible SAM and biologically modified substrates.

Cultures of E 12, 13, 14 neuronal cells, analyzed for both neurotransmitter and sub-population expression, as well as survival and longevity; and Microglia are being examined for response to substrate. These experiments are being compiled in manuscripts. Reports on these cultures will be forthcoming in next quarter's results.

#### *Collaborations*

We have established a collaboration with W. Agnew at the Huntington Medical Research Institute. While we have not yet specifically modified electrodes, we have successfully modified the poly-silastic sheath that holds a series of microelectrodes in place along the spinal cord during implantation (Agnew et al., 1990). One problem we are focused on solving is that the sheath has glial scar buildup and adhesions that eventually displace the electrodes laterally (with adhesion) and vertically (due to buildup of tissue). We have contacted Dr Albert Lossinsky and will begin a new series of experiments once

the costs of the *in vivo* work is determined and Dr. Agnew's approval of the scope of the project is obtained.

In addition to collaborations with the Huntington Institute, we have contacted and are in the process of establishing studies of *in vivo* implantation of modified materials into cortex in animal models with Cordell Gross at the University of Vermont and Sharon Juliano At Uniform services University of the Health Sciences in Bethesda, MD.

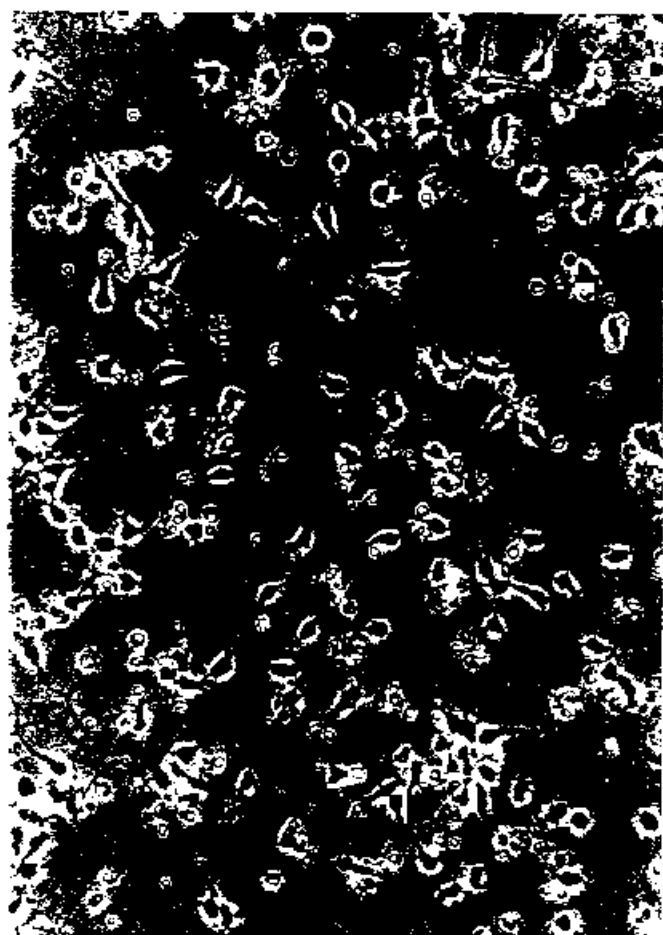
## NEXT QUARTER OBJECTIVES

- Extend screening of SAM surfaces for adult cultures
- Extend study of E12, E13, and E14 response to artificial surfaces
- Electrophysiologically examine adult and embryonic cultures on modified substrates
- Continue screening SAM surfaces for microglia response
- Continue screening biologically modified SAM surfaces
- Continue surface analysis of surfaces both before and after culture
- Send samples to University of Vermont for *in vitro* experiments as well as institute collaborative *in vivo* studies with USUHS

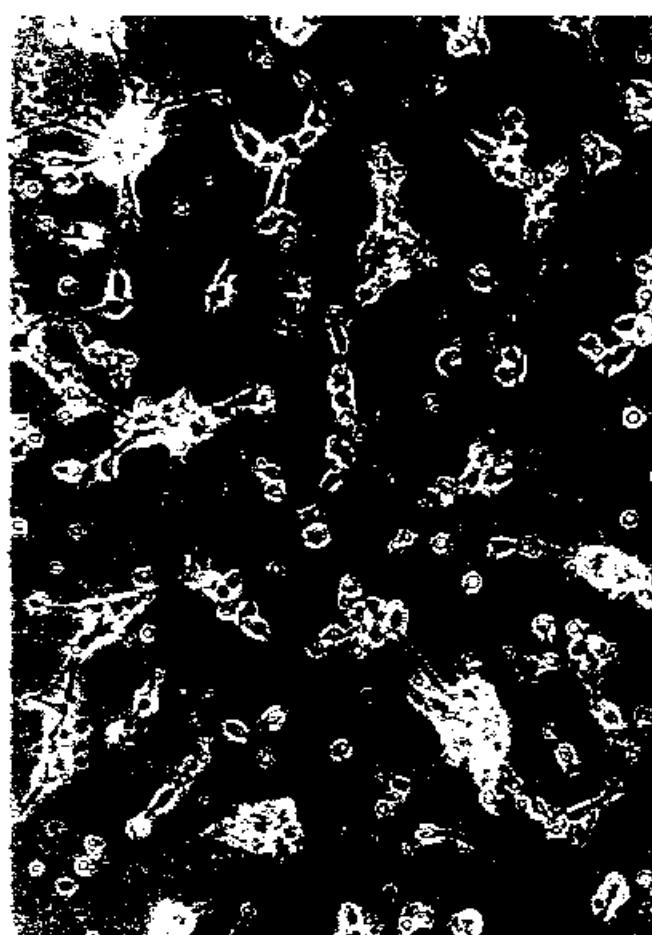
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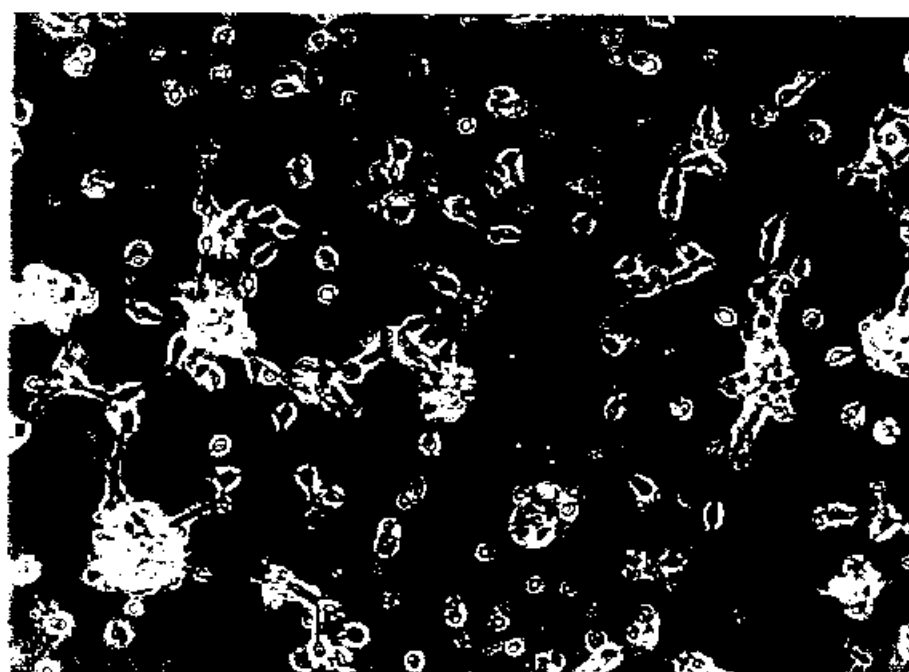
18 RAT CORTICAL NEURONS ON BIOLOGICALLY MODIFIED SUBSTRATES



A. PDL

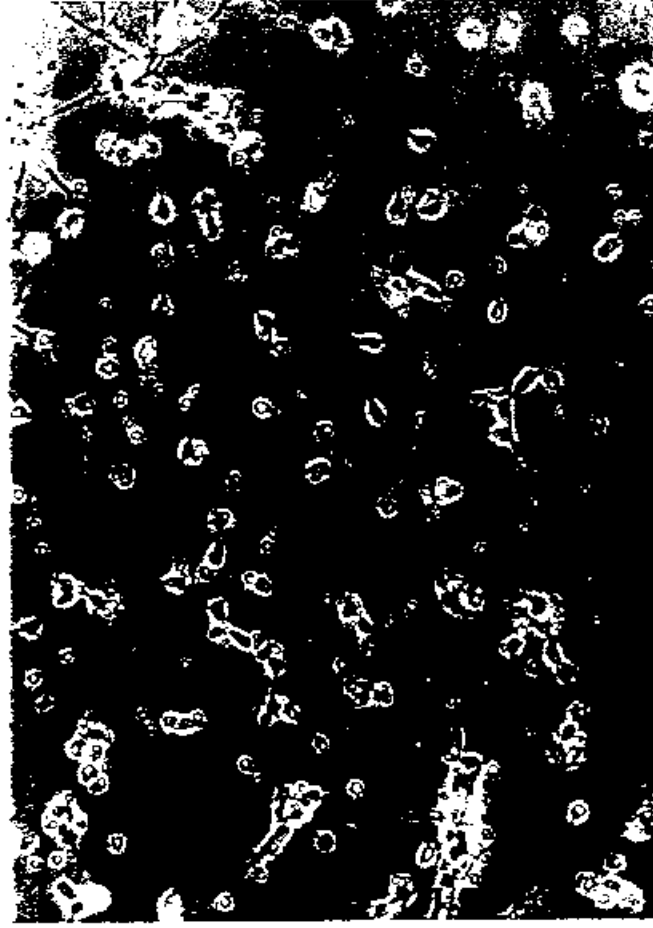


B. APTS

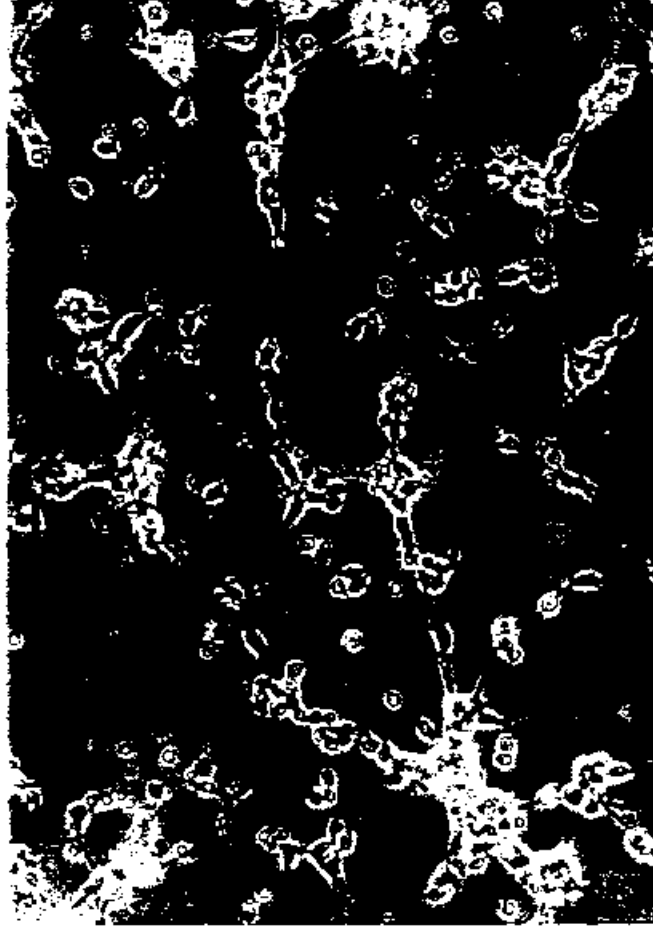


C. APTS + GLU

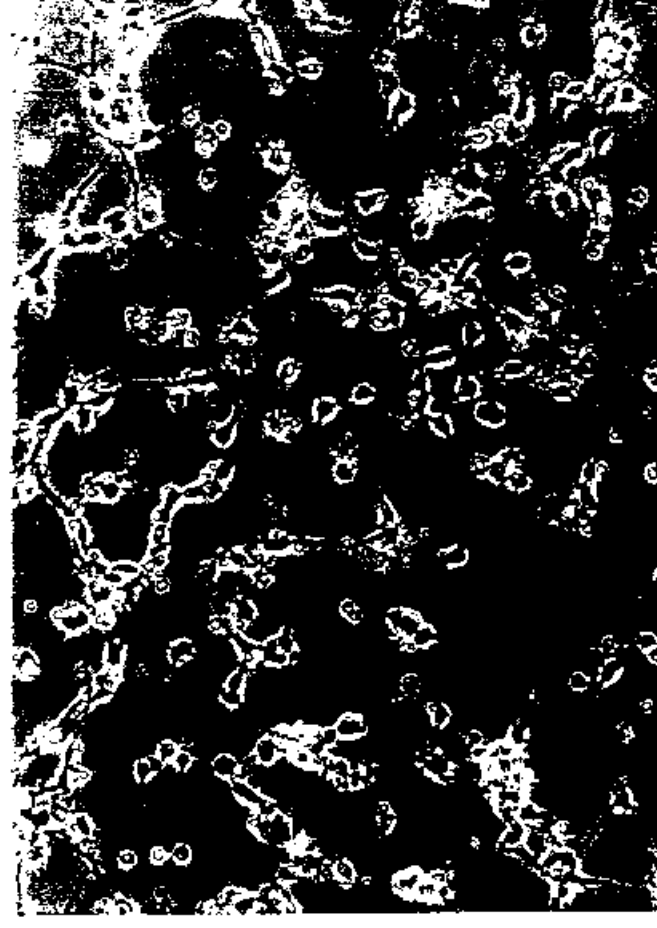
18 KAT CORTICAL NEURONS ON BIOLOGICALLY MODIFIED SUBSTRATES



A. APIS + APO



B. APIS + GLU + APO

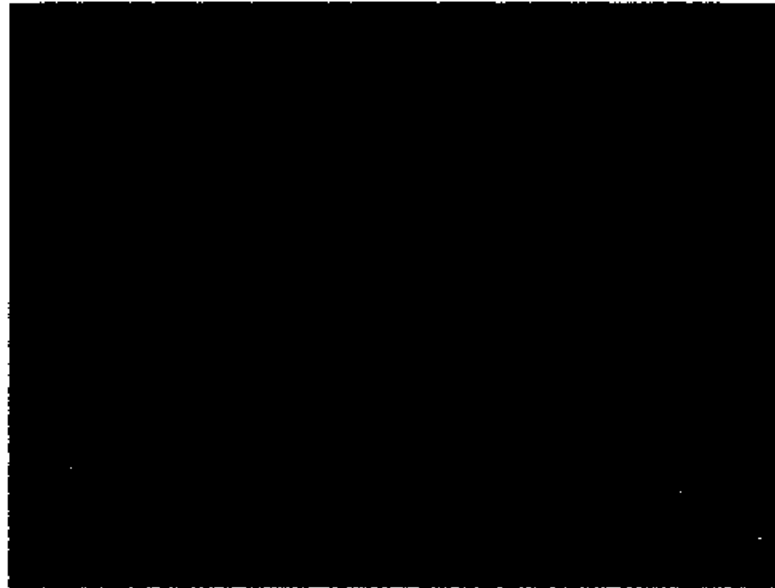


C. APIS + BSA

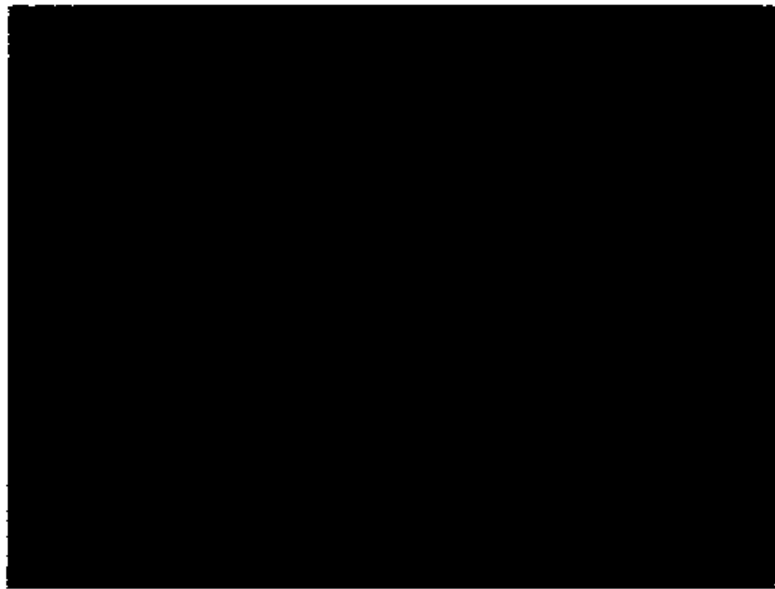


D. APIS + GLU + BSA

APTS/BSA

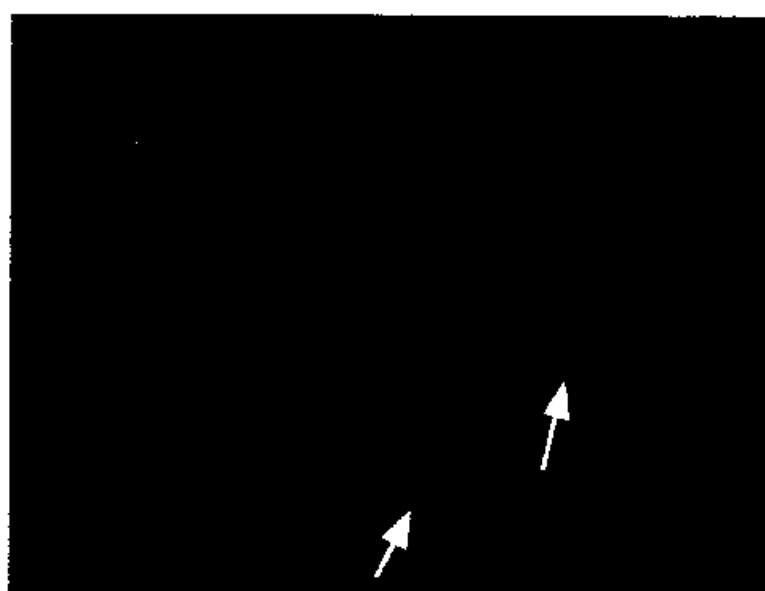


MAP2 Immunostaining

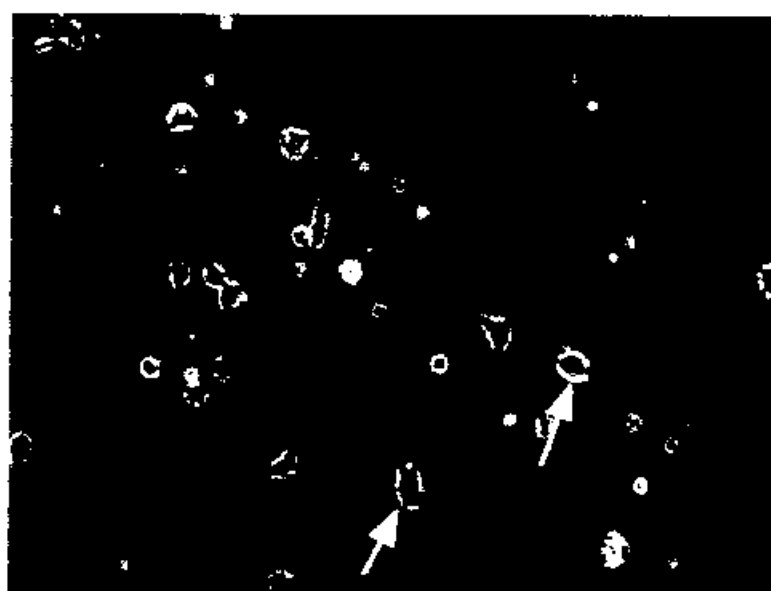


GFAP Immunostaining

APTS/G/BSA



GFAP immunostaining



Phase Contrast